8-Oxo-deoxyguanosine: Reduce, reuse, recycle?

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Despite a variety of antioxidant defenses, cellular production of oxidants such as reactive oxygen species leads to a background level of damage to the cell. Should the balance between oxidants and antioxidants shift in favor of the former, a condition of oxidative stress arises, which leads to widespread modification of molecules such as lipids and proteins. Nucleic acids, and their precursor (deoxy)ribonucleotide pools, are particular targets; >70 damage products have been described whose presence can have important implications for cell function (1). For example, in addition to producing mutation, oxidatively modified DNA can lead to alterations in cell signaling and gene expression, promote microsatellite instability, and accelerate telomere shortening (2). As a result, oxidative stress has been implicated in a wide variety of pathological conditions, including cancer, cardiovascular disease, aging, and neurodegenerative diseases (3). The most widely studied product of DNA oxidation is 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), along with its nucleobase equivalent 8-oxo-7,8-dihydroguanine (8-oxoGua). Well established methods are available for assessing this biomarker of oxidative stress in nuclear or mitochondrial DNA (4), as well as in extracellular matrices such as urine (5). The prevailing view is that extracellular 8-oxodG is principally a product of Nudix hydrolase and 5′-nucleotidase activities resulting from elimination of 8-oxodGTP from deoxyribonucleotide pools, whereas 8-oxoGua derives from the action of base excision repair enzymes such as human 8-oxoGua DNA glycosylase (hOGG1; Fig. 1). In a recent issue of PNAS, Hah *et al.* (6) used accelerator mass spectrometry, a technique with exquisite sensitivity, to experimentally examine previously difficult questions concerning the metabolic fate of 8-oxodG, using more realistic levels of substrates than were formerly feasible.

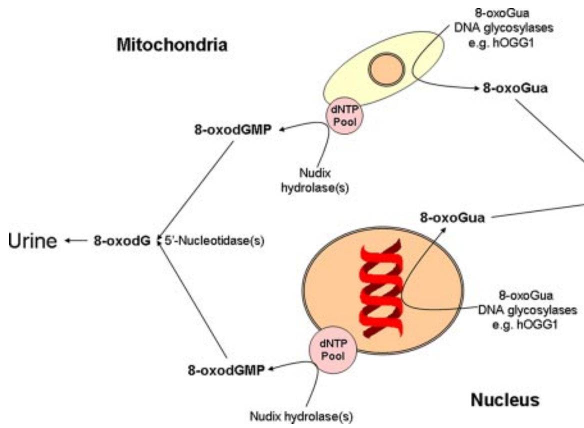

Fig. 1. Origin of extracellular 8-oxodG, and its nucleobase equivalent 8-oxoGua, through the action of Nudix hydrolase(s) toward the deoxyribonucleotide triphosphate (dNTP) pool and 8-oxoGua DNA glycosylases (e.g., hOGG1) toward DNA. (Modified from ref. 16.)

This work describes evidence for an apparently futile series of events in which extracellular 8-oxodG cycles through uptake, introduction into the deoxyribonucleotide pool, potential incorporation into nucleic acids or removal by Nudix hydrolases, with implied further processing by nucleotidases, and subsequent excretion into the extracellular milieu (Fig. 2). Such a finding is counterintuitive because, given the plethora of repair systems that exist to ensure that oxidatively modified bases do not persist in DNA, one would expect that DNA repair products, once removed, would not be substrates for reincorporation into nucleic acids.

The proposed salvage metabolism suggested by the authors is in some aspects in agreement, and in others at odds, with existing literature. Assuming that phosphorylation of 8-oxodGMP to 8-oxodGDP by guanylate kinase (normally involved in phosphorylation of GMP and dGMP to their corresponding dinucleotides) does not occur (7), the authors speculate on a route that circumvents this apparent impediment to the reutilization of 8-oxodG. This route hinges on the conversion of ribonucleotides to deoxyribonucleotides by ribonucleotide reductase, specifically, the conversion of 8-oxoGDP to 8-oxodGDP. However, in 1999, Hayakawa *et al.* (8) implied that ribonucleotide reductase may serve as a "gatekeeper" to specifically exclude the influx of species such as 8-oxoGDP into the 2′-deoxyribonucleotide pool, albeit not preventing their possible entry into RNA, the consequences of which are discussed elsewhere (9). It is therefore vital that the ability of ribonucleotide reductase to use 8-oxoGDP as a substrate is reevaluated. Two other enzyme activities important in the metabolic route suggested by the authors are purine nucleoside phosphorylase (PNPase) and hypoxanthine-guanine phosphoribosyltransferase (HGPRTase). Although HGPRTase is reported to be able to synthesize 8-oxoGMP from 8-oxoGua and 5-phospho-D-ribosyl-1-pyrophosphate (10), PNPase is reported to be inactive toward 8-oxodG (11). Therefore, as with ribonucleotide reductase, a critical reevaluation of the activity of PNPase toward 8-oxodG is warranted. The phosphorylation of 8-oxodG to 8-oxodGMP by deoxynucleoside kinase is reported not to occur (11) and, even if it were to do so, evidence suggests that no further metabolism of 8-oxodGMP would take place.

These recent findings may have profound implications for the measurement of 8-oxo(d)G, and potentially other DNA modifications, in both DNA and urine. It is conceivable that because 8-oxodG represents only a minor structural modification of deoxyguanosine, it can be utilized as a substrate for many endogenous metabolic pathways. However, it might be a significant oversight to assume that such a fate does not apply to other biomarkers of DNA damage, oxidatively derived or otherwise. Indeed, there is evidence to suggest that bromodeoxycytidine can be taken up by dividing cells and, after deamination and phosphorylation, and/or vice versa, is present in the dNTP pool as a substrate for DNA synthesis (12). A further consequence of the findings of Hah *et al.* (6) is to ascribe even greater importance to the activities of Nudix-type enzymes. These enzymes would appear to be the true gatekeepers ensuring that 8-

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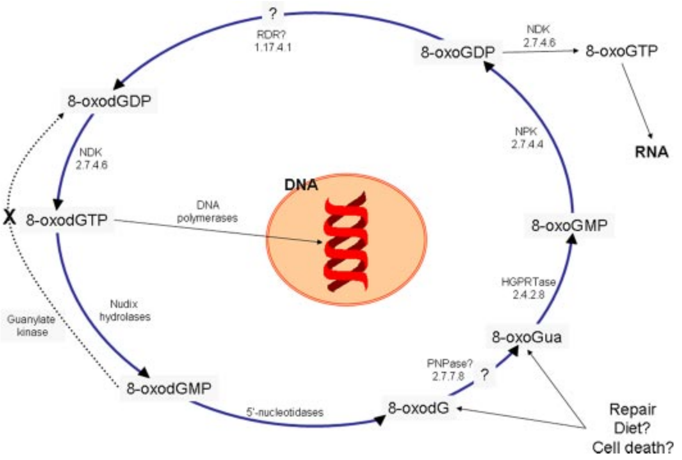

Fig. 2. Potential fate of extracellular 8-oxodG and 8-oxoGua via metabolic salvage pathways. Neither 8-oxodG nor 8-oxodGMP can be phosphorylated because they are not substrates for deoxynucleoside kinase or guanylate kinase, respectively. Therefore, the alternative pathway discussed by Hah *et al.* (6) relies on degradation of extracellular 8-oxodG to 8-oxoGua, perhaps by purine nucleoside phosphorylase (PNP). 6-Hydroxypurine phosphoribosyltransferase (HGPRTase) can catalyze the formation of 8-oxoGMP, providing a route for the oxidized moiety, via nucleoside-phosphate kinase (NPK) and nucleoside-diphosphate kinase (NDK), to be incorporated into RNA. In this model, ribonucleoside-diphosphate reductase (RDR) is responsible for the conversion of 8-oxoGua-containing ribonucleotides to deoxyribonucleotide equivalents. NDK then catalyses the phosphorylation of 8-oxodGDP to 8-oxoGTP, a substrate for DNA polymerases, for incorporation into DNA. Potential sources of extracellular 8-oxodG and 8-oxoGua (diet, cell death, DNA repair) are indicated, contributions from which may have profound implications for the cell and for our understanding of the true meaning of the measurement of these biomarkers of oxidative stress.

oxodGTP is excluded from the genome, irrespective of whether it is derived from the oxidation of dGTP *in situ* in the nucleotide pool or from the "piggy-backing" of extracellular 8-oxo(d)G onto the metabolic processes for native nucleobases and deoxyribonucleosides.

The authors also comment on a hypothesis that has received limited discussion or experimental examination in the literature: that of further oxidation of 8-oxodG. They provide interesting preliminary evidence that, under conditions of ongoing oxidative stress, 8-oxodG appears to be oxidized further. The chemical feasibility of this process has been known for some time, and the nature of several of the products has been identified. However, demonstration of their formation in a cellular system, in the context of free 2′-deoxyribonucleosides, has been lacking. Those laboratories that examine urinary 8-oxodG must now ask "How much material is missed because of further oxidation, and what is the implication of this for biomarker studies?"

Although it is certain that 8-oxodG is present extracellularly *in vivo*—having been measured in plasma (13) and cerebrospinal fluid (14), for example—its provenance is not entirely clear. In addition to the DNA repair process described above, cell turnover and the diet are possible sources of 8-oxodG and 8-oxoGua; although the contribution from these sources is considered to be minimal (15, 16), they have not been ruled out entirely. Indeed, significant contribution from the latter two routes probably negates the utility of measuring this lesion in urine, under any circumstances. The findings of Hah *et al.* (6) now extend this caveat to include assessment of nuclear, and potentially mitochondrial, 8-oxodG. Such measurements would, therefore, not be uniquely reflective of cellular oxidative stress but would nonetheless represent a DNA damage burden and hence a risk/threat to the cell.

If 8-oxodG is derived almost exclusively from DNA repair—either direct repair of DNA or, perhaps more likely, sanitization of nucleotide pools—then the possibility of urinary 8-oxodG being used as a phenotypic marker of selected repair activities is also likely to be out of the question if salvage or loss of 8-oxodG by further oxidation are significant processes. This, then, restores 8-oxodG to its original context as simply a generalized marker of oxidative stress.

Although the data of Hah *et al.* (6) certainly demonstrate the potential for extracellular 8-oxodG to be incorporated into cellular DNA, the references that we cite above add to the debate and give a fuller picture of the possible processes surrounding this interesting area. For researchers studying nucleic acid-derived biomarkers of oxidative stress, it is interesting to note that of 10.8 pmol of extracellular, radiolabeled 8-oxodG added to cells in culture, $\approx$8% was localized on or inside the cells. Of this $\approx$8%, only $\approx$1.0% became incorporated into DNA, with the remaining $\approx$92% being in the medium. Presumably this distribution reflects not only incorporation, but also removal, of radiocarbon-labeled 8-oxodG derivatives from DNA (and possibly RNA) and from the ribo- and deoxyribonucleotide triphosphate pools. Perturbation of these repair pathways could lead to greater incorporation into DNA. Other questions raised include the following. (*i*) To what extent does this phenomenon occur *in vivo*, especially in the body composed of largely nonreplicating cells (in which most incorporation is likely to be into RNA)? (*ii*) How big a contribution is salvage to 8-oxodGTP in the deoxyribonucleotide pool, compared with direct oxidation of dGTP (and other precursors), and what fraction of 8-oxodG in cellular DNA is derived from the deoxyribonucleotide pools after salvage? (*iii*) What are typical concentrations of extracellular or intracellular 8-oxodG?

Nevertheless, and of broadest significance, these data should be borne in mind when interpreting the measurement of 8-oxodG or 8-oxoGua in DNA and in extracellular matrices such as urine.

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